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# ISOCENTRATHERIN, A CYTOTOXIC GERMACRANOLIDE FROM *CENTRATHERUM PUNCTATUM* (COMPOSITAE)*

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**Key Word Index**—*Centratherum punctatum*; Compositae; germacranolide; isocentratherin; cytotoxicity.

**Abstract**—A cytotoxic germacranolide, isocentratherin, has been isolated from the aerial parts of *Centratherum punctatum* (Compositae).

## INTRODUCTION

Pursuant to our continuing search for anticancer principles of plant origin, a cytotoxic germacranolide, isocentratherin (1) has been isolated from the aerial parts of (*Centratherum punctatum* Cass.) (Compositae), native to Brazil.

## RESULTS AND DISCUSSION

Isocentratherin (**1**), mp 187–188° was obtained by monitoring the *in vitro* (KB assay system [2]) activity of the chloroform-soluble fraction of the aerial parts of *C. punctatum*. Elemental analysis and accurate mass measurement established the molecular formula to be $C_{20}H_{22}O_7$. A combination of IR, UV and $^1H$ NMR spectral data indicated the presence of a $\gamma$-lactone with an exocyclic methylene group ($\nu_{max}$ 1760 and 1660 $cm^{-1}$, $\lambda_{max}$ 210 nm $\varepsilon$ 19 200, $\delta$ 5.48 and 6.20, d's, $J = 3$ Hz) and an angelate ester ($\nu_{max}$ 1720 $cm^{-1}$, $\delta$ 6.2, q, $J = 6.5$ Hz coupled to a 3 H d at 1.87, and fragment ions $[M - C_5H_7O]^+$ and $[M - C_5H_7O - H_2O]^+$. A secondary allylic alcohol was suspected ($\nu_{max}$ 3350 $cm^{-1}$, $\delta$ 3.74, br s) and confirmed when acetylation produced a monoacetate **2**, mp 149°.

Also supported from the spectral data was the presence of an extended dihydrofuran-3-one system similar to that present in centratherin (**3**) [3], goyazensolide (**4**) [4] and related compounds [5]. Thus IR bands were observed at 1690, 1635 and 1595 $cm^{-1}$ [3, 4], the UV spectrum displayed a maximum at 269 nm ($\varepsilon$ 8550), and in the $^1H$ NMR spectrum a sharp singlet was observed at $\delta$ 5.85 for H-2 of the furanone ring. But of special significance were the characteristic carbon resonances for C-1, C-2, C-3, C-8 and C-10 (Table 1), which are substantially in agreement with those established previously for identical molecular arrays [4] and serve to distinguish the C-6,12 $\gamma$-lactone isomers. The SFORD $^{13}C$ NMR spectrum also indicated the presence of two exomethylene groups ($\delta$ 124.0 and 126.7), the second of which should involve C-4(15). These functionalities, in a germacranolide ring system, are in accord with the established molecular formula.

In the $^1H$ NMR spectrum of the acetate the $\alpha$-allylic methine proton was deshielded to $\delta$ 6.18 and one of the protons assigned to C-15 of the C-4(15) exomethylene moiety was shifted from $\delta$ 5.92 to 6.08; implying isocentratherin to be the allylic alcohol **1**, isomeric to centratherin (**3**).

The $^1H$ NMR spectral assignments and certain stereochemical aspects of isocentratherin were established by double resonance experiments at 270 MHz. Irradiation at the frequency of H-7 collapsed the doublets at $\delta$ 5.48 (H-13) and 6.20 (H-13) to sharp singlets and also reduced the multiplicity of the broadened doublet at 4.82, assigned to H-6. Irradiation at 4.31 reduced the triplet ($J = 12.8$ Hz) at 2.55 to a doublet, indicating the latter to be the H-9$\alpha$ proton *trans*-diaxial to H-8 $\beta$. Irradiation of the doublet ($J = 12.4$ Hz) at 2.34 perturbed the H-9 signal at 2.55, but did not simplify the H-8 resonance, suggesting a dihedral angle close to 90° between H-8 and H-9. The multiplicity of H-6 ($J = 5.2, 0.6$ Hz) implies that the angle between H-5 and H-6 is also close to 90° and that the C-5 and C-6 constituents are both $\alpha$.

All of the available spectral data are in accord with the proposed structure for isocentratherin. The acetate of this isolate, namely **2**, should be identical with the acetate of centratherin [3], but unfortunately no direct comparison of the two samples could be made. We are in the process of establishing the structure of isocentratherin by single crystal X-ray crystallography.

Ohno *et al.* [3] obtained centratherin (**3**) as the major sesquiterpene lactone of the leaves and stems of *C. punctatum*. Our compound is not an artefact of isolation, for it is one of the major compounds present in the methanol extract and does not accumulate during processing or chromatographic purification.

Isocentratherin (**1**, NSC-295429) was active ($ED_{50}$ 0.30 μg/ml) in the KB cytotoxicity test [2], but was inactive in the P-338 test system *in vivo*, at doses in the range 2.5–25.0 mg/kg.

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*Part XVII in the series "Potential Anticancer Agents". For Part XVI see ref. [1].

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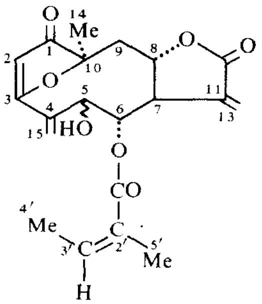
Table 1. $^{13}$C NMR spectral data for isocentratherin (**1**), goyazensolide (**4**) and goyazensolide acetate (**5**)*

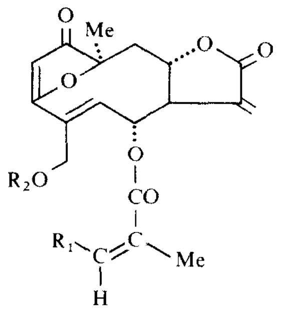
| Carbon | Isocentratherin (**1**) | Goyazensolide (**4**)† | Goyazensolide acetate (**5**)† |
|---|---|---|---|
| 1 | 198.7 | 204.6 | 204.0 |
| 2 | 106.6 | 106.4 | 107.0 |
| 3 | 185.7 | 184.6 | 185.1 |
| 4 | 137.7 | 135.6 | 134.0 |
| 5 | 75.3 | 134.6 | 75.0 |
| 6 | 85.9 | 81.7 | 83.8 |
| 7 | 44.5 | 51.0 | 46.2 |
| 8 | 71.4 | 73.5 | 72.1 |
| 9 | 44.5 | 43.3 | 44.7 |
| 10 | 90.4 | 89.7 | 90.4 |
| 11 | 134.0 | 135.6 | 134.0 |
| 12 | 167.1 | 166.8 | 166.9 |
| 13 | 126.7 | 126.2 | 126.5 |
| 14 | 21.2 | 20.6 | 21.1 |
| 15 | 124.0 | 62.9 | 130.6 |
| 1′ | 169.4 | 168.7 | 168.2 |
| 2′ | 126.3 | | |
| 3′ | 140.5 | | |
| 4′ | 15.6 | | |
| 5′ | 19.9 | | |

*Recorded in $CDCl_3$ on a Bruker HX-90E instrument at 22.63 MHz, with TMS as internal standard.

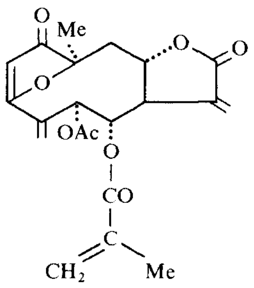
†Data are from Vichenewski *et al.* [4].



**1**



**2** $R_1$ = Me; $R_2$ = Ac
**3** $R_1$ = Me; $R_2$ = H
**4** $R_1$ = $R_2$ = H



**5**

Table 2. $^1$H NMR spectral data for isocentratherin (**1**), centratherin (**3**) and goyazensolide (**4**)

| Proton | Isocentratherin (**1**)* | Centratherin (**3**)† | Goyazensolide (**4**)*‡ |
|---|---|---|---|
| 2 | 5.85, *s* | 5.86, *s* | 5.83, *s* |
| 5 | 4.89, *m* | 6.32, *dt* (1.5, 1.5, 5) | 6.27, *dt* (1.5, 1.5) |
| 6 | 4.82, *br d* (5.2, 0.6) | 5.48, *m* (1.5, 1.5, 2.5) | 5.33, *dt* (2.5, 1.3) |
| 7 | 4.23, *m* | 3.85, *m* (3.3, 3.2, 5) | 3.80, *m* (3, 3, 3, 2.5, 2.5) |
| 8 | 4.31, *d* (12.2) | 4.58, *dt* (10, 3.3) | 4.53, *dt* (2.5, 13) |
| 9 | 2.55, *t* (12.4) | 2.58, *dd* (13, 10) | 2.50, *dd* (1.5, 2.5) |
| 9 | 2.34, *d* (12.8) | 2.30, *dd* (13, 3) | 2.30, *dd* (15, 3) |
| 13 | 5.48, *d* (3.0) | 5.52, *d* (3) | 5.49, *d* (3) |
| 13 | 6.25, *d* (3.2) | 6.27, *d* (3) | 6.22, *d* (3.3) |
| 14 | 1.55, *s* | 1.52, *s* | |
| 15 | 5.91, *s* | | |
| 15 | 5.94, *s* | | |
| 3′ | 6.19, *q* | 6.2, *m* (1.2, 8) | |
| 4′ | 1.87, *d* (6.5) | 1.90, *m* (2) | 1.83 |
| 5′ | 1.78, *s* | 1.80, *dq* (1.8) | |
| OH | 3.74, *s* | | |

*Recorded in $CDCl_3$ on a Bruker 270 MHz instrument with TMS as an internal standard. Coupling constants (Hz) are given in parentheses.

†Data are from Ohno *et al.* [3]. Recorded in $CDCl_3$ on a Varian HA-100 instrument with TMS as an internal standard.

‡Data are from Vichenewski *et al.* [4].

## EXPERIMENTAL

*Plant material.* The aerial parts of *Centratherum punctatum* Cass. (Compositae) were collected in Brazil in 1974. Identification was made by Dr. A. Barclay of the Economic Botany Laboratory, Agricultural Research Service, BARC-East, U.S.D.A., Beltsville, MD.

*Preliminary extraction and isolation.* The dried, ground aerial parts of *C. punctatum* (40.4 kg) were successively extracted with petrol (3 × 30 l.) and MeOH (3 × 45 l.). After evapn, the MeOH extract was partitioned between $CHCl_3$ (30 l.) and water (15 l.), to afford, after processing, 1 kg of $CHCl_3$ extract. A sample (19.5 g) of this extract was subjected to CC on Si gel PF-254 (600 g). Isocentratherin (200 mg, 0.025 %) crystallized from fractions eluted with $CHCl_3$–3 % MeOH, mp 187–188°, $[\alpha]_D^{25}$ $-28.5°$ (*c*, 0.50, $CHCl_3$). UV $\lambda_{max}^{MeOH}$ nm (log ε): 210 (4.283) and 269 nm (3.932). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3350, 1760, 1710, 1660, 1640, 1590. $^1$H NMR, Table 2; $^{13}$C NMR, Table 1. MS *m/z* (rel. int.): 374 [M$^+$] (10), 356 (8), 291 (6), 274 (8), 83 (100) and 55 (10). (Found: C, 63.69; H, 5.87; Calc. for $C_{20}H_{22}O_7$: C, 64.16; H, 5.91 %). Mass measurement, Found: 374.1368; Calc. for $C_{20}H_{22}O_7$: 374.1365.

*Acetylation of isocentratherin.* Isocentratherin (10 mg) was treated with pyridine–$Ac_2O$ (5 ml, 20:1) under $N_2$ at room temp. for 2 hr. After solvent removal *in vacuo*, the residue was washed with cold 2 N HCl (2 ml) and extracted into $Et_2O$ (3 × 15 ml). The $Et_2O$ extract was washed with satd NaCl, dried ($Na_2SO_4$) and evapd. Crystallization of the residue from MeOH afforded **2** as white needles (5.9 mg), mp 149°. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1760, 1740, 1710, 1660, 1590. $^1$H NMR ($CDCl_3$): δ 1.55 (3 H, *s*, H-14), 1.80 (3 H, *s*, H-5), 1.85 (3 H, *d*, $J = 6.5$ Hz, H-4), 2.09 (3 H, *s*, OAc), 2.34 (1 H, *d*, $J = 12.8$ Hz, Hβ-9), 2.55 (1 H, *dd*, $J = 12.4$ Hz, Hα-9), 4.18 (1 H, *s*, H-1), 4.31 (1 H, *d*, $J = 12.2$ Hz, H-8), 4.90 (1 H, *d*, $J = 5.4$ Hz, H-6), 5.51 (1 H, *d*, H-13), 5.87 (1 H, *s*, H-2), 5.93 (1 H, *br s*, H-15), 6.08 (1 H, *br s*, H-15), 6.18 (1 H, *br s*, H-5) and 6.25 (1 H, *d*, H-13). MS *m/z* (rel. int.): 416 [M$^+$] (15), 373 (5), 356 (15), 291 (20), 274 (8), 83 (100), 55 (10) and 43 (70).

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